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Relationship of Specific Bacteria in the Cervical and Vaginal Microbiotas with Cervicitis

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Abstract

Background—Cervicitis is an inflammatory condition of the cervix associated with upper genital tract infection and reproductive complications. Although cervicitis can be caused by several known pathogens, the etiology frequently remains obscure. Here we investigate vaginal bacteria associated with bacterial vaginosis as potential causes of cervicitis.

Methods—Associations between vaginal bacteria and cervicitis were assessed in a retrospective case control study of women attending a Seattle STD clinic. Individual bacterial species were detected using two molecular methods: quantitative PCR (qPCR) and broad range 16S rRNA gene PCR with pyrosequencing. The primary finding from this initial study was evaluated using qPCR in a second cohort of Kenyan women.

Results—The presence of *Mageeibacillus indolicus*, formerly BVAB3, in the cervix was associated with cervicitis, while the presence of *Lactobacillus jensenii* was inversely associated. Quantities of these bacteria did not differ between cervicitis cases and controls, though in a model inclusive of presence and abundance, *M. indolicus* remained significantly associated with cervicitis after adjustment for other cervicitis-causing pathogens. *M. indolicus* was not associated with cervicitis in our study of Kenyan women, possibly due to differences in the clinical definition of cervicitis.

Conclusions—Colonization of the endocervix with *M. indolicus* may contribute to the clinical manifestations of cervicitis, but further study is needed to determine whether this finding is

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repeatable and applicable to diverse groups of women. Colonization of the cervix with *L. jensenii* could be a marker of health, perhaps reducing inflammation or inhibiting pathogenic infection.

Keywords

cervicitis; bacterial vaginosis; BVAB

INTRODUCTION

Cervicitis, presenting as inflammation of the uterine cervix, is a syndrome usually caused by infection. Cervicitis has classically been associated with *Chlamydia trachomatis* or *Neisseria gonorrhoeae* infection[1], but can also accompany *Trichomonas vaginalis*, *Mycoplasma genitalium*, and herpes simplex virus infection[2, 3]. However, more than half of cervicitis cases lack known etiology[4]. Clinical signs of cervicitis include purulent endocervical exudate, increased numbers of polymorphonuclear leukocytes (PMNs) in the cervix, and easily induced cervical bleeding (friability)[2]. Cervicitis is a predictor of upper genital tract inflammation[5] and has been associated with poor pregnancy outcomes[6] and increased HIV-1 shedding[7].

Intriguing evidence points to a link between cervicitis and bacterial vaginosis (BV)[8, 9]. BV is a complex polymicrobial infection[10] associated with early pregnancy loss and preterm birth[11], as well as pelvic inflammatory disease[12]. In the largest study of BV and cervicitis to date, 15% of 423 women with BV had coincident cervicitis and the majority of these women (87%) had no conventional pathogen detected[13]. In a separate study of women with both BV and cervicitis, adding treatment for BV in the form of metronidazole vaginal gel to a standard regimen of doxycycline and ofloxacin improved cure rates for cervicitis[14]. These data suggest that some BV-associated bacteria may contribute to cervicitis.

Detection of fastidious urogenital bacterial species with quantitative PCR (qPCR) has been associated with preterm birth[15], persistent BV[16], and urethritis in men[17]. Here we applied this methodology to investigate the relationship of eight BV-associated bacterial species/genera and three of the most common vaginal lactobacilli with cervicitis in a retrospective case control study of women attending a Seattle STD clinic. We also examined the cervical microbiotas of these women by deeply sequencing broad-range 16S rRNA gene amplicons. Our findings suggest the cervical microbiota is not markedly altered in women with cervicitis, but the presence of certain species in the cervix may be associated with clinical manifestations of cervicitis.

MATERIALS AND METHODS

Clinical Procedures

Our overarching approach was to explore associations of bacterial species with cervicitis in one cohort and subsequently confirm or refute our findings using a separate cohort. The Fred Hutchinson Cancer Research Center institutional review board approved this protocol and written informed consent was obtained from all participants. For the initial study we recruited women age 18 years and older seeking health care for a new problem at the

Seattle-King County STD clinic between September 2006 and June 2010. Women were interviewed regarding their medical and sexual history prior to undergoing a standardized pelvic exam with speculum. After collecting a vaginal swab specimen from the lateral wall, visible adherent discharge was removed from the ectocervix with a large cotton swab prior to inserting a second swab into the endocervical canal for sample collection. The endocervical swab was rolled onto a sterile glass slide for Gram staining and both swabs were stored at −80° C until DNA extraction. Assessment of sexually transmitted infections (STI) is described in the **Supplemental Methods** provided as **Supplemental Digital Content** (**SDC_Text**).

Cohort Selection

Our Seattle study enrolled 238 women, 210 of whom had complete clinical data and a cervical exam. From these 210 women, 14 cervicitis cases (6.7%) were identified according to the following criteria: 1) purulent exudate visible in the endocervical canal or on an endocervical swab specimen; or 2) presence of cervical friability with sustained bleeding of the endocervix upon gentle passage of a swab through the cervical os; or 3) an average of 30 or more polymorphonuclear leukocytes (PMNs) per high power field on endocervical Gram stain. Of the remaining 196 women, 8 were excluded due to missing STI infection data. Eligible controls were further limited to those who enrolled within 140 days of a given case and matched on DNA extraction method prior to random selection of 3 controls for each case.

Molecular Analyses

DNA extraction is described in the **Supplemental Methods** (**SDC_Text**). Taxon-directed quantitative PCR assays targeting common vaginal bacteria and those with high specificity for BV were employed along with a PCR inhibition control as previously described[10, 18]. The lower limit of detection for qPCR runs was 2.5 to 10 gene copies per reaction, with the exception of three runs with a detection limit of 100 gene copies per reaction. Excluding these lower sensitivity runs from the analysis had no effect on our conclusions. No-template PCR and sham DNA extraction controls were run with each PCR assay to monitor for contamination.

To detect other cervical bacteria, cervical DNA samples from each case and 2 of its 3 controls were subjected to broad-range 16S rRNA gene PCR and the resulting amplicons sequenced with 454 Life Sciences Titanium pyrosequencing technology (Roche, Branford, CT)[19, 20]. Samples were multiplexed using 6 bp barcodes (**SDC_Table_S1**). We generated 337,303 reads with an average of 8,031 reads/sample (SRP058048). However, owing to low DNA concentration, only 456 reads were generated for one cervicitis case, (p7z1tr80). Read sequences were classified using the *pplacer* [21] phylogenetic placement tool with a curated reference set of vaginal bacteria[19]; 97.8% of the reads were classified to the species level.

Kenyan Study

A nested case control cohort was drawn from a longitudinal open cohort study of high-risk women in Mombasa, Kenya. Women 16 years or older who presented to the clinic and reported engaging in transactional sex were eligible to enroll. Women provided written

informed consent for a protocol approved by the institutional review boards at the Kenyatta National Hospital (Nairobi, Kenya), the University of Washington, and the Fred Hutchinson Cancer Research Center. Visits consisted of a face-to-face interview to collect information on medical and sexual history, followed by a speculum-assisted pelvic examination. Cervicitis was diagnosed by observation of mucopurulent discharge at the cervical os or ≥30 PMN counts per high power field on endocervical Gram stain, as described above; data on friability were not collected. Of 608 women considered for the study, 603 had complete data on mucopus and cervical PMN counts; 21 (3.5%) of these women had cervicitis. Three controls were randomly chosen for each case from the remaining 582 eligible participants. STI detection and DNA extraction information is provided in the **Supplemental Methods** (**SDC_Text**).

Statistical Analyses

Bivariate associations were assessed using Fisher's exact tests and Wilcoxon rank sum tests. Due to small sample size, regression analyses were performed using exact logistic regression with p-values and confidence intervals calculated using the sufficient statistic. Statistical analyses were completed using Stata SE 13.1 (Stata, College Station, Texas) and all p-values reported at the 95% confidence level. The Venn diagram was constructed using eulerAPE[22]. To visualize the pyrosequencing data, Euclidean distances between asinh-transformed ('inverse hyperbolic sin' transforms data with near-zero values) sequence read abundances were hierarchically clustered using average linkage. Lasso regression with binomial response was used to model the relationship between asinh-transformed species abundance and cervicitis status in R's *glmnet* package[23]. This statistical technique forces some parameter estimates to zero, identifying a subset of taxa influencing cervicitis.

RESULTS

Seattle STD Clinic Study

Seattle study participants were predominately young Caucasian women with a low prevalence of genital infections; there were no significant differences between women with and without cervicitis with regards to race, ethnicity, sexual behaviors, or the presence of co-infections (p >0.1, **Table 1**). Women with cervicitis were more likely to have *Mageeibacillus indolicus*, formerly BVAB3, detected in the cervix (42.9% vs. 11.9%, p=0.020) and in the vagina (42.9% vs. 16.7%, p=0.067) than women without cervicitis (**Table 2**). In addition, there was a trend towards an association between detection of *Megasphaera* species type 2 in the cervix and cervicitis (28.6% vs. 7.1%, p=0.058). *Lactobacillus jensenii* was the only lactobacillus species inversely associated with cervicitis, detected in the cervix of 52.4% of women without cervicitis and only 14.3% of women with cervicitis (p=0.015). No statistically significant associations between cervicitis and the concentration of bacterial species were observed among women with detectable levels of the species (**Figures S1–S2** in **SDC_Text**); 16S and 18S rRNA gene copy numbers (indicators of sampling error) also showed no association with cervicitis (data not shown).

Although our small study size precluded extensive multivariable analysis, we performed exact logistic regression to assess associations between the presence and quantity of specific

bacteria and cervicitis, adjusting for the presence of *Chlamydia trachomatis* (CT), *Neisseria gonorrhoeae* (GC), and *Trichomonas vaginalis* (TV) (**Table 3)**. We modeled the risk of cervicitis with 10-fold increments of 16S rRNA gene copies in the cervix, assigning samples in which the bacterium was not detected a value of half the lower limit of detection; in this way both the presence and load of each bacterial species contributed to the model. After adjusting for STIs, each 10-fold increase of *M. indolicus* bacterial load in the cervix and vagina was associated with a 53% (p=0.064) and 63% (p=0.019) increased risk of cervicitis, respectively.

To further examine the role of the cervical microbiota in cervicitis, we deeply sequenced broad-range 16S rRNA gene amplicons from a subset of our Seattle cohort. Unsupervised hierarchical clustering separated cervical bacterial communities by BV status (Nugent score), but no cluster groups were associated with cervicitis case/control status (**Figure 1**). We also examined associations of bacteria with cervicitis using penalized linear regression models to determine linkages between cervicitis and specific bacterial species, including those not targeted by qPCR. A single bacterium, *M. indolicus*, had a positive association with cervicitis (regression coefficient 0.175). No bacterium had a negative association in the model. No reads were classified as the cervicitis-associated bacterium *Mycoplasma genitalium*, though we cannot completely rule out the possibility that a single nucleotide mismatch between the *M. genitalium* 16S rRNA gene sequence and one of our 16S broad range primers may have impacted our ability to detect this organism.

Kenyan Study

We subsequently examined whether *M. indolicus* was associated with cervicitis in a separate case control cohort of Kenyan women that included 21 cervicitis cases. Like in the Seattle cohort, detection of cervicitis-causing pathogens was relatively uncommon, although 40.5% of the women in our Kenyan cohort were HIV positive (**Table 4**). The prevalence of BV was lower in the Kenyan cohort than in the Seattle cohort (Amsel 15.7%; Nugent 33.3%), but as shown in **Table 4**, these and other clinical characteristics did not differ between groups of women with and without cervicitis.

By qPCR, *M. indolicus* was present in over 55% of vaginal samples obtained from Kenyan women, compared to 23% of those from Seattle women. However, there was no significant difference in the frequency with which *M. indolicus* was detected vaginally in women with and without cervicitis (52.4% vs. 57.1%; p=0.800; **Table 4**). There was also no association between the presence of *M. indolicus* and cervicitis in an exact logistic regression model adjusted for DNA extraction method and the presence of other cervicitis-causing infections (aOR = 0.74, 0.24–2.32, p=0.611). Finally, there was no difference in *M. indolicus* bacterial load in Kenyan women with and without cervicitis (**Figure S3** in **SDC_Text**).

We hypothesized that one possible reason *M. indolicus* was not associated with cervicitis in the Kenyan study was that cervicitis was defined differently in the two studies. Because data on cervical friability were not available for the Kenyan women, we reevaluated the Seattle STD clinic data with this clinical marker of cervicitis factored separately (**Figure 2**). Upon refitting our multivariate model, we found *M. indolicus* was not significantly associated with

mucopus or high cervical PMN counts in the Seattle STD clinic (aOR 2.53, 0.40–14.43, p=0.228; **Table 5**). *M. indolicus* was, however, significantly associated with cervical friability (aOR 8.24, 1.25– 63.89, p=0.013).

DISCUSSION

This study explored novel bacterial etiologies of cervicitis using two molecular approaches: qPCR to measure presence and concentration of key vaginal bacteria and broad-range 16S rRNA gene PCR with pyrosequencing for more comprehensive species-level profiling. Cervicitis was present in 7% of women attending a Seattle STD clinic. While BV was common among women diagnosed with cervicitis, only 4 out of 14 cervicitis cases were associated with other identifiable STIs (CT, GC, TV). Our qPCR studies linked detection and increasing load of the BV-associated bacterial species *M. indolicus* with cervicitis. Pyrosequencing of broad-range PCR amplicons from cervicitis cases and a subset of controls confirmed the association of *M. indolicus* with cervicitis using a model that accounted for multiple comparisons.

M. indolicus is a *Clostridiales* order bacterium[24] that has been linked to treatment failure in women with BV[25]. It was recently detected in men with non-gonoccocal urethritis (NGU) along with *Megasphaera* spp., *Leptotrichia/Sneathia* spp., and *Atopobium* spp[17]. In that study, *M. indolicus* was associated with report of prior episodes of NGU and STI. Together these data support the hypothesis that this bacterium may be sexually transmitted, potentially playing a pathogenic role in both the female and male genitourinary tracts.

Association of *M. indolicus* with cervicitis was not observed in the cohort of Kenyan women we examined, suggesting this finding may not be generalizable to all women. One reason for the lack of consistency may be that women of African origin appear to have higher carriage rates of many BV-associated bacteria compared to Caucasian women[19]. In Seattle studies with relatively few (<25%) African American women, the prevalence of *M. indolicus* was 16–31%[18, 25]. In contrast, in our Kenyan cohort and in a New Orleans study with 95% of the participants identifying as African American, the prevalence of *M. indolicus* was 56–57% [26]. Although *M. indolicus* is often detected in women with BV and BV is typically more common in African and African-American women than Caucasian women[27], this does not explain the higher prevalence of *M. indolicus* in our Kenyan cohort since it had a lower prevalence of BV than our Seattle cohort. We speculate that *M. indolicus* might be a relatively common member of the vaginal microbiota of Kenyan women due to race-related genetic differences that allow for higher immunological tolerance of the organism and perhaps a diminished role in inflammatory conditions like cervicitis. Another possible explanation for the discrepancy in our results stems from the behavioral practices of the women in our Kenyan study. As these women are sex workers who are known to engage in frequent vaginal washing practices[28], it is possible that some of the bacteria detected in cross-sectional analysis were recently acquired and/or transient; this would reduce our ability to detect disease associations in this cohort.

A third factor likely contributing to the different results obtained in the Seattle and Kenyan studies are differing clinical definitions of cervicitis. Inspection of the relationship of *M.*

indolicus with cervicitis-defining clinical criteria in the Seattle cohort failed to identify significant association with neutrophilic infiltrate in the form of visible mucopus or elevated PMN counts. *M. indolicus* was significantly associated with friability, but this clinical marker was not recorded in the Kenyan study. There are several reasons the cervix may become prone to bleeding, including cervical edema, vascular swelling, and erosion. With regard to cervicitis, these changes are generally thought to occur as part of the inflammatory response to a cervical pathogen; thus friability is often causally linked to mucopus and high numbers of PMNs. *M. indolicus* could affect the tendency of cervical tissue to bleed in the absence of a neutrophilic immune response. The periodontis-associated microbe *Porphyromonas gingivalis* is thought to directly contribute to gum bleeding during infection of gingival tissue by degrading fibrinogen to prevent blood coagulation[29]. If our data can be confirmed, it would be of interest to determine whether *M. indolicus* similarly encodes virulence factors that alter coagulation or vascular permeability; an equally plausible and testable hypothesis is that cervical bleeding promotes the growth of *M. indolicus*.

L. jensenii is the only species we observed having a protective effect against cervicitis and mucopus/high PMN counts. Shimazu et al. demonstrated *L. jensenii* strain TL2937 attenuates the inflammatory response mediated by toll-like receptors (TLRs) by altering the expression of both upstream and downstream signaling molecules, including NF-κB, in a porcine intestinal cell culture model[30]. The present study provokes the question of whether vaginal strains of *L. jensenii* may also deactivate the innate immune response and reduce cervical inflammation, or simply provide colonization resistance against BV-associated bacteria.

This study is not the first to examine the role of the cervical and vaginal microbiotas in cervicitis, but it is the first to link particular BV-associated species to the condition. Ling et al. previously examined the role of *G. vaginalis*, *A. vaginae*, *Eggerthella*, *Prevotella*, *Leptotrichia/Sneathia*, and *Megasphaera* species type 1 in cervicitis using PCR-denaturing gradient gel electrophoresis (PCR-DGGE) and qPCR[31]. In their Chinese cohort of 30 healthy women and 70 women with BV and/or cervicitis, no significant associations of these microbes with cervicitis were identified. Ling et al. did observe *Lactobacillus* genus was inversely associated with cervicitis, but associations with individual *Lactobacillus* species (*L. crispatus*, *L. jensenii*, and *L. iners*) did not achieve significance.

Our study has several strengths, including our approach of coupling the sensitivity of qPCR data with the exploratory breadth of 16S rRNA gene PCR with pyrosequencing. In addition, our primary study surveyed both the endocervix and vagina; although we and others found the microbiota at these different anatomical sites to be similar[31], the observed associations between bacterial species and cervicitis were often more significant in cervical specimens and might have been missed had we only analyzed vaginal swabs. Finally, we employed NAATs and cultivation to ensure we controlled for the presence of most other infectious causes of cervicitis.

Our study also has limitations. Foremost is the low prevalence of cervicitis in both cohorts, which limited our power to detect associations with cervicitis. Second, our ability to detect *M. genitalium* by 16S rRNA gene PCR may have been limited by primer mismatch and we

did not survey for viral causes of cervicitis such as HSV and cytomegalovirus (CMV). Third, we did not consistently collect information on hormonal birth control and thus were not able to adjust for this potential confounder[13]. Although our findings suggest *M. indolicus* could be associated with idiopathic cervicitis, additional epidemiological studies are needed to confirm this finding and increase our understanding of how microbes may interact with the host to produce clinical indicators of cervicitis.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Short summary

A case control study of women attending an STD clinic in Seattle identified associations between the BV-associated *bacterium Mageeibacillus indolicus* and clinical indicators of cervicitis.

Figure 1.

Heat map of bacterial abundances in association with bacterial vaginosis and clinical indicators of cervicitis. Dendrogram shows association of meta data with vaginal bacterial communities. Nugent score for bacterial vaginosis and cervicitis-defining clinical criteria are expressed as negative (light) and positive (dark). The most abundant bacterial taxa (top 30%) are shown (see **SDC_Table_S2** for complete data). PMNs = polymorphonuclear leukocytes.

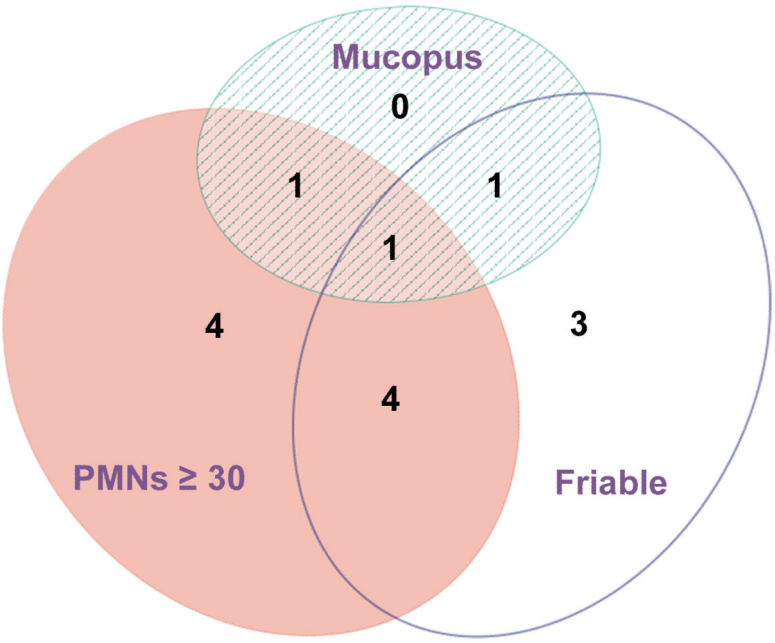

Figure 2.

Area-proportional Venn diagram depicting the individual clinical criteria used to define cervicitis in the Seattle cohort.

Table 1

Clinical and Behavioral Characteristics of Seattle Participants With and Without Cervicitis*

Characteristic	Cervicitis Present N=14	Cervicitis Absent N=42	All Participants N=56
Age (years)			
Median (IQR)	26.5 (21–32)	27 (24–33)	27 (23–32)
Range	19–46	17–57	17–57
Race			
Black/African American	5 (35.7%)	8 (19.1%)	13 (23.2%)
Caucasian	5 (35.7%)	28 (66.7%)	33 (58.9%)
Other	3 (21.4%)	3 (7.1%)	6 (10.7%)
Not known/provided	1 (7.1%)	3 (7.1%)	4 (7.1%)
Ethnicity			
Hispanic	2 (14.3%)	4 (9.5%)	6 (10.7%)
Not hispanic	12 (85.7%)	36 (85.7%)	48 (85.7%)
Not known/provided	0	2 (4.8%)	2(3.6%)
Male sex partners			
New partner, past 60 days	2 (14.3%)	11 (26.2%)	13 (23.2%)
≥ 1sex partner, past 90 days	12 (85.7%)	33 (78.6%)	43 (23.2%)
Median # (IQR), past 90 days	1 (1 – 1)	1 (1 – 2)	1 (1–1.5)
Female sex partners			
New partner, past 60 days	0	1 (2.4%)	1 (1.8%)
Any, past 90 days	2 (14.3%)	4 (9.5%)	6 (10.7%)
Used condom at last vaginal sex	4 (28.6%)	15 (35.7%)	19 (33.9%)
Ever douched	8 (57.1%)	20 (47.6%)	28 (50.0%)
Took oral antibiotics, past month	0	4 (9.5%)	4 (7.1%)
Co-infections			
Chlamydia trachomatis	1 (7.1%)	1 (2.4%)	2 (3.6%)
Neisseria gonorrhoeae	1 (7.1%)	0	1 (1.8%)
Trichomonas vaginalis	2 (14.3%)	4 (9.5%)	6 (10.7%)
Vaginal candidiasis	0	8 (19.1%)	8 (14.3%)
Bacterial vaginosis			
Amsel criteria	8 (57.1%)	15 (35.7%)	23 (41.1%)
Nugent score			
0 – 3 – Negative	3 (21.4%)	21 (50.0%)	24 (42.9%)
4 – 6 – Intermediate	1 (7.1%)	2 (4.8%)	3 (5.4%)
7 – 10 – Positive	10 (71.4%)	19 (45.2%)	29 (51.79%)

* $p > 0.1$ for all tests (Wilcoxon rank sum test for age and Fisher exact tests for categorical variables) comparing cases and controls.

Table 2

Presence of BV-Associated Bacteria and Lactobacilli in Seattle Participants With and Without Cervicitis

	Detection in the Cervix n (%)			Detection in the Vagina n (%)		
BV-associated Bacteria	**Cervicitis Present N=14**	**Cervicitis Absent N=42**	**p-value***	**Cervicitis Present N=14**	**Cervicitis Absent N=42**	**p-value***
Gardnerella vaginalis	12 (85.7)	34 (81.0)	1.000	13 (92.9)	36 (85.7)	0.666
Atopobium vaginae	12 (85.7)	25 (59.5)	0.106	12 (85.7)	29 (69.1)	0.307
Leptotrichia/Sneathia	9 (64.3)	21 (50.0)	0.537	8 (57.1)	22 (52.4)	1.000
Megasphaera type 1	8 (57.1)	17 (40.5)	0.357	9 (64.3)	19 (45.2)	0.355
Megasphaera type 2	4 (28.6)	3 (7.1)	0.058	4 (28.6)	5 (11.9)	0.206
BVAB1	3 (21.4)	6(14.3)	0.676	3 (21.4)	9 (21.4)	1.000
BVAB2	8 (57.1)	15 (35.7)	0.213	9 (64.3)	16 (38.1)	0.123
Mageeibacillus indolicus	6 (42.9)	5 (11.9)	0.020	6 (42.9)	7 (16.7)	0.067
Lactobacilli						
Lactobacillus crispatus	4 (28.6)	20 (47.6)	0.350	8 (57.1)	22 (52.4)	1.000
Lactobacillus jensenii	2 (14.3)	22 (52.4)	0.015	3 (21.4)	19 (45.2)	0.205
Lactobacillus iners	12 (85.7)	39 (92.9)	0.590	14 (100.0)	39 (92.9)	0.565

BV = bacterial vaginosis.

* Fisher exact tests. p-values were not adjusted for multiple comparisons.

Table 3

Multivariate Analyses of the Relationship Between the Presence and Load of Specific Bacterial Species in Seattle Participants and Cervicitis

Presence Alone	**Detection in the Cervix**		**Detection in the Vagina**	
	aOR (95% CI)[†]	**p-value**[*]	**aOR (95% CI)**[†]	**p-value**[*]
Mageeibacillus indolicus	4.38 (0.84–23.68)	0.086	2.93 (0.57–14.78)	0.232
Lactobacillus jensenii	0.17 (0.02–0.89)	0.032	0.41 (0.06–2.17)	0.401

Presence and Load[‡]	**Detection in the Cervix**		**Detection in the Vagina**	
	aOR (95% CI)[†]	**p-value**[*]	**aOR (95% CI)**[†]	**p-value**[*]
Mageeibacillus indolicus	1.53 (0.98–2.44)	0.064	1.63 (1.08–2.56)	0.019
Lactobacillus jensenii	0.61 (0.29–1.02)	0.061	0.68 (0.40–1.02)	0.068

[†]Each bacterial species was modeled independently as they do not always co-occur. Each model was adjusted for the presence of *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, and *Trichomonas vaginalis*, but not multiple comparisons.

[*]Calculated using exact logistic regression with p-values reported by the sufficient statistic.

[‡]Odds ratio interpreted as increase in odds of cervicitis for each 10-fold increase in bacterial load (16S copies) for each individual species. Models include all 56 subjects; those in which the species in question was not detected were assigned a value of half the lower limit of detection. As such, the differential prevalence and load of each species in women with and without cervicitis influences the odds ratios shown.

Table 4

Characteristics of Women and Presence of Mageeibacillus indolicus in Kenyan Participants With and Without Cervicitis*

Characteristic	Cervicitis Present N=21	Cervicitis Absent N=63	All Participants N=84
Age (years)			
Median (IQR)	29 (26-33)	29 (25-34)	29 (21-42)
Range	22-41	19-46	19-46
HIV Positive	7 (33.3%)	27 (42.9%)	34 (40.5%)
Co-infections			
Chlamydia trachomatis	0	1 (1.6%)	1 (1.2%)
Neisseria gonorrhoeae	1 (4.8%)	2 (3.2%)	3 (3.6%)
Trichomonas vaginalis	4 (19.1%)	5 (7.9%)	9 (10.7%)
Vaginal candidiasis	5 (23.8%)	10 (15.9%)	15 (17.9%)
Bacterial vaginosis			
Amsel criteria	2 (9.5%)	11 (17.7%)	13 (15.7%)
Nugent score(≥7)	4 (19.1%)	24 (38.1%)	28 (33.3%)

Bacteria	Cervicitis Present N=21	Cervicitis Absent N=63	p-value†
Mageeibacillus indolicus			
Vaginal detection	11 (52.4%)	36 (57.1%)	0.80

*p > 0.1 for all tests (Wilcoxon rank sum test for age and Fisher exact tests for categorical variables) comparing cases and controls.

†Fisher exact test.

Table 5

Relationship between the Presence of Cervicitis-Associated Species in the Cervix and Clinical Criteria Used to Define Cervicitis in the Seattle Study

	Friability		Mucopus or PMNs ≥ 30	
BV-associated Bacteria	**aOR (95% CI)†**	**p-value***	**aOR (95% CI)†**	**p-value***
Mageeibacillus indolicus	8.24 (1.25-63.89)	0.013	2.53 (0.40-14.43)	0.228
Lactobacilli				
Lactobacillus jensenii	0.35 (0.03-2.06)	0.279	0.11 (0.00-0.85)	0.018

BV = bacterial vaginosis; PMNs = polymorphonuclear leukocytes.

† Each bacterial species was modeled independently as they do not always co-occur. Each model was adjusted for the presence of *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, and *Trichomonas vaginalis.*

* Calculated using exact logistic regression with a conditional probability test.